

GEORGIA INSTITUTE OF TECHNOLOGY  
OFFICE OF CONTRACT ADMINISTRATION  
SPONSORED PROJECT INITIATION

Date: 10/3/80

Project Title: Stereological Analysis of Biological Soft Tissues

Project No: E-23-654

Project Director: Raymond P. Vito

Sponsor: Biomedical Research Support Grant (G-32-608)

Agreement Period: From June 16, 1980 Until March 31, 1981

Type Agreement: DHEW/PHS/NIH Grant No. 2 S07 RR07024-15 (\$ 81,771 awarded for 15th year)

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Technical Matters

Contractual Matters

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Defense Priority Rating: None

Assigned to: Engineering Science and Mechanics (School/~~Laboratory~~)

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Other Dr. John W. Crenshaw, Jr.  
File G-32-608

GEORGIA INSTITUTE OF TECHNOLOGY  
OFFICE OF CONTRACT ADMINISTRATION  
SPONSORED PROJECT TERMINATION

Date: May 18, 1981

Project Title: Stereological Analysis of Biological Soft Tissues

Project No: E-23-654

Project Director: Raymond P. Vito

Sponsor: DHEW/PHS/NIH; Biomedical Research Support Grant (G-32-608)

Effective Termination Date: 3/31/81

Clearance of Accounting Charges: 3/31/81

Grant/Contract Closeout Actions Remaining:

NONE

- ☐ Final Invoice and Closing Documents
- ☐ Final Fiscal Report
- ☐ Final Report of Inventions
- ☐ Govt. Property Inventory & Related Certificate
- ☐ Classified Material Certificate
- ☐ Other \_\_\_\_\_

Assigned to: ESM (School/Laboratory)

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Other G-32-608

E-23-654

under G-32-608

Sterological Analysis of Biological  
Soft Tissues  
Final Report

by

Raymond P. Vito  
Associate Professor  
April 30, 1981

## Introduction

This report is a summary of work done under Biomedical Research Support Grant G-32-608.

The Grant was used to purchase equipment and supplies needed for the image analysis of histological sections of biological soft tissues. This report summarizes the algorithm developed for this purpose and the progress made in implementing the algorithm.

## Algorithm

It is well known that any color can be generated by a superposition of the primary colors<sup>\*</sup> red, blue and green. Mathematically,

$$I_A = C_1 I_R + C_2 I_B + C_3 I_G$$

where  $I_A$  represents the intensity of light at a particular wavelength (color) A,  $I_R$ ,  $I_B$  and  $I_G$  represent light intensities of red, blue and green light respectively and  $C_1$ ,  $C_2$  and  $C_3$  are constants.

The TV image digitizer generates a number between zero and 255 which corresponds to the light intensity at each of the 640 X 480 points, or "pixels", in the field of view. Mathematically, a picture may be thought of as a two-dimensional array of intensities  $I(J,K)$   $J = 1, \dots, 480$ ,  $K = 1, \dots, 640$ . One begins by digitizing three pictures using either a red, a blue or a green filter. Delectric filters, which have a very narrow band-pass, are used. The pictures  $I_R(J,K)$ ,  $I_B(J,K)$ ,  $I_G(J,K)$ , are stored on a disk. They are also normalized to account for the different attenuations of the filters and the fact that the microscope light source is not true white light. This is done by removing the slide and adjusting the light level so as to achieve the same measured light intensity with each filter.

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<sup>\*</sup>Note: these are really red, blue and yellow; but green is adequate for our purposes.

It follows from equation (1) that one may imagine the color of pixel (J,K) to be represented by a point in the "color space" shown in Figure (1). One can now manually scan the field of view

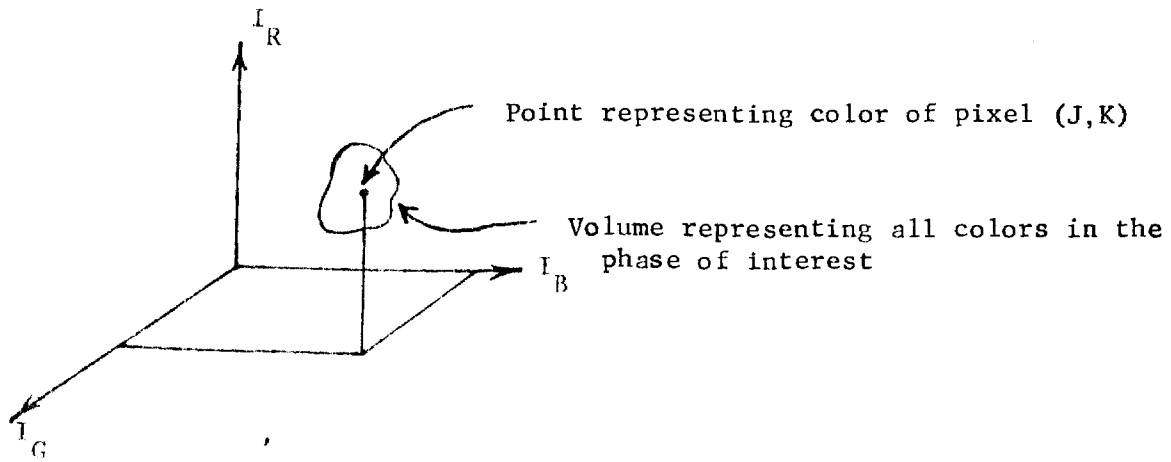


Figure 1

using a joystick cursor and record the (J,K) coordinates of a number of pixels representative of those in the phase of interest. These coordinates may be stored on a disk. The computer now automatically searches through pictures  $I_R(J,K)$ ,  $I_B(J,K)$ , and  $I_G(J,K)$  and determines the intensity values which correspond to the colors of these pixels. These intensity values define a series of points in the color space of Figure (1). Given a sufficient number of points, one may think of the points as defining a volume in such a way that any pixel in the phase of interest has a color which lies in the volume.

Once the computer has been "trained" to recognize the colors in the phase of interest, it can automatically scan the pictures  $I_R(J,K)$ ,  $I_B(J,K)$  and  $I_G(J,K)$  and record all the (J,K) coordinates of the pixels in the phase of interest. These coordinates provide the basis for the stereological analysis discussed below. They may be stored in a disk or they

may be used to generate and display a black/white picture showing clearly the separated phase. Note that once the volume in color space corresponding to the phase of interest is adequately defined, one can proceed to analyze other fields of view or other slides without additional manual input.

### Progress

Two major\* FORTRAN programs have been written and tested. These are briefly described below.

POINTS This program requests the user to select (using a joystick cursor) a number of points in the phase of interest. It outputs a set of co-ordinates (x,y) which may be read by program PHASE.

PHASE This program requires three digitized (and normalized) pictures  $P_0$ ,  $P_1$  and  $P_2$  taken through red, blue and green filters. The sets of co-ordinates (x,y) determined by POINTS are used to search  $P_0$ ,  $P_1$ ,  $P_2$  for the red, blue and green intensities at each point. These define a volume in "color space" which corresponds to the phase of interest. The pictures  $P_0$ ,  $P_1$  and  $P_2$  are then used to generate a fourth picture which is black in the phase of interest and white elsewhere. This black/white picture is available for image analysis (areas, orientations, etc.).

### Application

The algorithm is now working and various tests are being run. Specifically, we are investigating

- a) the shape and size of the sample volume and how it affects the results

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\*Additional small programs were written but are not documented here.

- b) whether or not the sum of the separated phases gives  
the original picture (internal check)
- c) the choice of filters for the best results

The author's primary interest is the arterial wall and a proposal dealing with this was submitted to the Georgia Heart Association.

However, arterial structure is extremely complex. We are, therefore, currently focusing on the "Reid Index" (Reid 1960) which involves an area measurement of mucus secreting bronchial cells. This simple problem permits checking the automatic analysis with a manual one.

This work is in progress, will be reported in the literature and will acknowledge the support of DHEW.

#### Acknowledgement

I'd like to thank Ms. Mary Bordonaro for her help in implementing this algorithm.

#### Reference

Reid, Lynne; Measurement of the Bronchial Mucous Gland Layer: a diagnostic yardstick in chronic bronchitis. Thorax 15 (1960)